

REMARKS

I. 35 U.S.C. §103

The Examiner has rejected Claims 28-34, 36, 46, 48, 49, and 52-55 under 35 U.S.C. §103 as unpatentable over Mehta et al., WO 00/07018 ("Mehta et al.") in view of Keenan et al., *Bioorg. Med. Chem.*, 6: 1309-1335 (1998 ("Keenan et al.")).

According to the Examiner,

"Keenan et al. teach a method comprising the steps of (i) providing a hybrid ligand such as dimerized FK1012 derivative linked by polyethylene linkers, (ii) introducing the hybrid ligand into a population of cells containing a SEAP reporter gene operably linked to ZFHD1 binding sequences, a first chimeric gene encoding a fusion protein containing three FKBP binding domains and a DNA binding domain from FHD1, and a second chimeric gene encoding a fusion polypeptide containing three FKBP binding domains and a transcription activation domain from the NF- κ B p65 subunit, and (iii) allowing the hybrid ligand to bind the FKBP binding domains to induce dimerization such that transcription of the SEAP reporter gene is increased, and (iv) identifying positive ligand binding cells by activation of SEAP." (See, Office Action, paragraph bridging pages 5 and 6.)

* * *

"Because both Mehta et al. and Keenan et al. teach methods of using a hybrid ligand to activate gene expression in a cell, and because Mehta et al. teach it is within the skill of the art to use any known linker to link ligand A and ligand B, it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute the PEG linker of 3 or 4 units of Kennan [sic] et al. for the hydrocarbon linker of Mehta et al." (See, Office Action, page 6.)

Keenan et al. fails to teach a screening method to identify a polypeptide that binds to a user-specified ligand, as is recited in Claim 28. As disclosed in Keenan et al., a number of ligands with varying linkers and/or varying binding monomers were tested for their relative abilities to dimerize known binding partners for the monomers. Keenan et al. are looking for the linker-monomer combination that gives the best dimerization of a known binding partner. Any screening is for a linker and/or monomer, not any binding partner (e.g., the candidate ligand binding domain of Claim 28) that binds the monomer.

Keenan et al. never teach or suggest a candidate ligand-binding domain P2 as in step (ii)(c) of Claim 28, since both ligand-binding domains taught in Keenan et al. are already known

and invariable. Keenan et al. fail to teach the screening of a library of nucleic acid sequences (encoding candidate P2).

In addition, Applicants assert that Keenan et al. do not perform the step according to step (v) of Claim 28 as they do not perform any actual screening.

Therefore, Keenan et al. fail to teach at least the candidate ligand-binding domain P2 and step (v) of Claim 28.

Also, in contrast to the Examiner's assertion, the Keenan et al. document teaches away from PEG linkers (e.g., 1q-1r) as recited in Claim 28, because the Keenan et al. article indicates that the ability of these PEG linkers to "induce apoptosis in the Fas assay was poor". (See, Keenan et al., page 1313, second column, second full paragraph.) In Keenan et al., a variety of linkers were screened to see if any of them would outperform the hybrid ligand, 1d, which is the reference compound with an apoptosis IC_{50} of 6 nM, and transcription assay EC_{50} of 15 nM (transient transfection assay) or 20 nM (stable transfection assay). (See, Keenan et al., page 1311, Table 1.) Compounds 1i-1s each share the same FKBP12-binding monomer as that of 1d, but differ in their respective linker sequences. (See, Table 1, pages 1311-1312, right column, page 1313, to left column, page 1314.) Based on these assays, Keenan et al. conclude that the three polyether linkers tested in ligands 1p-1r, described as "[a] more radically altered set of compounds" (see, page 1313, right column), are "poor" in terms of their ability to induce apoptosis.

For example, the reference linker in 1d has an IC_{50} of about 6 nM. In contrast, the best of 1p-1r has an IC_{50} of about 140 nM, about 24 times worse than 1d. The same linkers, 1p-1r, are also considerably worse than 1d in both the stable and the transient transfection assay, with EC_{50} between 5-20 times worse than that of 1d. Overall, among the 11 linkers similarly tested, all but one, 1o, are better than the polyether linkers in both the apoptosis assay and the transient transfection assay. (See, Keenan et al., Table 1.)

For the foregoing reasons, the combination of Mehta et al. with Keenan et al., does not render obvious Claims 28-34, 36, 46, 48, 49, and 52-55.

Reconsideration and allowance of Claims 28-34, 36, 46, 48, 49, and 52-55 are respectfully requested.

II. 35 U.S.C. §103

The Examiner has rejected Claims 28-34, 36, 46, 48, 49, and 52-55 under 35 U.S.C. §103 as unpatentable over Mehta et al. *supra*, in view of Bertozzi et al., *J. Org. Chem.*, 56: 4326-4329 (1991) ("Bertozzi et al.").

According to the Examiner,

"Mehta et al. teach a three-hybrid method for identifying the targets such as proteins of biologically active small molecules, where multiple proteins are screened for interactions with any small ligand . . . Mehta et al. do not teach the method where the linker of the hybrid ligand comprising ligand A (R1) and ligand B (R2) has the formula $(CH_2-O-CH_2)_n$, where n is an integer from 2 to 5." (See, Office Action, pages 7 and 9.)

With respect to Bertozzi et al., the Examiner states,

"Bertozzi et al. teach that polyethylene glycol derivatives are ideal for the purpose of linking two compounds to make bifunctional molecules for the study of enzymes and receptors, because they are inexpensive, water soluble, and available in a variety of lengths . . . Bertozzi et al. teach that the heterobifunctional linker [disclosed by Bertozzi et al.] contains a free amine that can be conjugated to biological molecules directly by an amide linkage (or via the corresponding isothiocyanate) and an azide that can be reduced to an amine for conjugation to other molecules . . ." (See, Office Action, page 9.)

In conclusion, the Examiner states,

"It would have been obvious to one of ordinary skill in the art at the time the invention was made to replace the linker of the hybrid ligand of Mehta et al. with the linker taught by Bertozzi et al., because both references teach the use of a linker to link two moieties. Thus, it would have been obvious to one skilled in the art to substitute one linker for another to achieve the predictable result of linking the two moieties for use in the screening assay." (See, Office Action, page 10.)

Applicants assert that Bertozzi et al. teach away from using PEG linkers of the present invention. Specifically, Bertozzi et al. disclose the use of PEG as a linker moiety in heterodimeric hybrid ligands with increased water solubility (hydrophilicity), as well as the chemistry and synthesis of such ligands. Bertozzi et al. emphasize that PEG linkers are water-soluble and often highly so. (See, Bertozzi et al., page 4326, right column, line 2.) Therefore, as is well known in the art, biological membranes are highly hydrophobic, i.e., not hydrophilic, and thus, when attempting to provide improved membrane-permeable heterodimeric hybrid ligands

useful for *in vivo* application, the skilled artisan would be motivated to search for hydrophobic moieties, rather than hydrophilic moieties. In other words, the skilled artisan would have had no motivation to use ligands incorporating such a linker for *in vivo* use, because the PEG linkers are known to be hydrophilic, and therefore would be expected to decrease membrane permeability of the ligand.

The combination of the teachings of Bertozzi et al. added to the disclosure of the use of a hydrocarbon linker by Mehta et al. to link hybrid ligands, fails to render obvious Claims 28-34, 36, 46, 48, 49, and 52-55.

The present invention discloses the surprising discovery that the use of PEG linkers in accordance with the teachings disclosed herein, actually increases the cellular uptake of the hybrid ligands, as shown in Figures 6 and 7 and Example 7 of the present specification. This discovery is surprising in view of the Bertozzi et al. teaching and the known water solubility of polyethylene glycols.

For the foregoing reasons, reconsideration and allowance of Claims 28-34, 36, 46, 48, 49, and 52-55 are respectfully requested.

III. 35 U.S.C. §103

The Examiner has rejected Claims 43-45 under 35 U.S.C. §103 as unpatentable over Johnsson et al., U.S. Pat. No. 5,585, 245 ("Johnsson et al."), in view of Licitra et al., *Proc. Natl. Acad. Sci., USA*, 93: 12817-12821 (1996) ("Licitra et al."), and Bertozzi et al., *supra*, as evidenced by Varshavsky et al., *Proc. Natl. Acad. Sci., USA*, 93: 12142-12149 (1996) ("Varshavsky et al.").

According to the Examiner,

"It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the two-hybrid method of Johnsson et al. to include the hybrid ligand, P1 and P2 portions taught by Licitra et al. because Licitra suggest the use of other two- or three- hybrid systems to expand the utility of the assay comprising the hybrid ligand and Johnsson et al. teaches a version of a three-hybrid method. Further, it would have been obvious to one of ordinary skill in the art at the time the invention was made to replace the linker of the hybrid ligand of Licitra et al. with the linker taught by Bertozzi et al., because both references teach the use of a linker to link two moieties." (See, Office Action, page 13.)

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"Varshavsky is cited only to show that N-end rule degradation operates in all organisms examined, from mammals to fungi and bacteria." (See, Office Action, page 12.)

As stated previously, Applicants amended Claims 43 and 44 to recite a specific linker "Y" structure, e.g., PEG linker, which is not taught or suggested in Johnsson et al. or Licitra et al. In addition, Licitra et al. teach away from improving the linker in their hybrid ligand by suggesting that a better approach would be to "generate yeast strains that are more permeable without significantly affecting yeast viability." (See, Licitra et al., page 12820, right column.)

Therefore, the combination of Johnsson et al., Licitra et al., Bertozzi et al., and Varshavsky et al. can not render obvious Claims 43-45.

Reconsideration and allowance of Claims 43-45 are respectfully requested.

IV. 35 U.S.C. §103

The Examiner has rejected Claims 28-34, 36, 46, 48-50, 52, and 53 under 35 U.S.C. §103 as unpatentable over Liu et al., U.S. Pat. No. 5,928,868 ("Liu et al."), in view of Bertozzi et al., *supra*.

According to the Examiner,

"It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the hybrid ligand of the three-hybrid method of Liu et al. to include the linker comprising a free amine and azide of Bertozzi et al. because Liu et al. teach it is within the ordinary skill in the art to use any method known in the art to link ligands A and B to form a hybrid molecule and Bertozzi et al. teach the use of the linker to form a hybrid ligand." (See, Office Action, paragraph bridging pages 16 and 17.)

Liu et al. describe a cell-based *in vivo* screening method using hybrid ligands. Improved membrane permeability of the heterodimeric ligands is of utmost importance for such *in vivo* application to proceed. As is well known in the art, biological membranes are highly hydrophobic (i.e., not hydrophilic) and, as such, when attempting to provide improved membrane-permeable heterodimeric hybrid ligands useful for *in vivo* application, such as the screening assay disclosed in Liu et al., a skilled artisan would be motivated to look for hydrophobic moieties, rather than hydrophilic moieties, such as those PEG linkers disclosed in Bertozzi et al. In other words, a skilled artisan would have had no motivation to use ligands incorporating such a linker for *in vivo* use, because the PEG linkers are known to be hydrophilic, and therefore would be expected to

decrease membrane permeability of the ligand. Even if the PEG linker provided some benefit to link moieties A and B of the hybrid ligand taught by Liu et al., such benefit is certainly outweighed by the paramount importance of membrane permeability required of such hybrid ligand.

As stated above, the present invention discloses the surprising discovery that the use of PEG linkers in accordance with the teachings disclosed herein, actually increases the cellular uptake of the hybrid ligands, as shown in Figures 6 and 7 and Example 7 of the present specification.

Therefore, the combination of Liu et al. and Bertozzi et al. can not render obvious Claims 28-34, 36, 46, 48-50, 52, and 53.

Reconsideration and allowance of Claims 28-34, 36, 46, 48-50, 52, and 53 are respectfully requested.

V. 35 U.S.C. §103

The Examiner has rejected Claims 28-36, 46, 48-50, 52, and 53 under 35 U.S.C. §103 as unpatentable over Liu et al. *supra*, in view of Bertozzi et al. *supra*, and Lin et al., *J. Amer. Chem. Soc.*, 122: 4247-4248 (2000) ("Lin et al.").

According to the Examiner,

"[I]t would have been obvious at the time the invention was made to modify the hybrid ligand of Liu et al. to include methotrexate as A (or R1), because Liu et al. teach that A can be varied and Lin et al. teach the use of methotrexate in a three-hybrid assay." (See, Office Action, page 19.)

Lin et al. does not teach a hybrid ligand R1-Y-R2, wherein R2 binds or inhibits a kinase. Applicant respectfully asserts that the Examiner is basing this rejection on the erroneous assumption that methotrexate is a kinase inhibitor, R2. Even if dexamethasone (Dex) in Lin et al. is interpreted as "R2", it binds glucocorticoid receptor (GR), a nuclear transcription factor.

Therefore, the combination of Liu et al., Bertozzi et al., and Lin et al. can not render obvious Claims 28-36, 46, 48-50, 52, and 53.

Reconsideration and allowance of Claims 28-36, 46, 48-50, 52, and 53 are respectfully requested.

VI. 35 U.S.C. §103

The Examiner has rejected Claims 28-34, 36, 46, and 48-53 under 35 U.S.C. §103 as unpatentable over Liu et al. *supra*, in view of Bertozzi et al. *supra*, and Karlsson et al., U.S. Pat. No. 6,143,574 ("Karlsson et al.").

According to the Examiner,

"[I]t would have been obvious at the time the invention was made to include the use of plasmon resonance to determine the binding affinity of A to P1, because Liu et al. teach it is within the skill of the art to select A and P1 based upon binding affinity and Karlsson et al. teach a method of determining binding affinity using plasmon resonance." (See, Office Action, paragraph bridging pages 21 and 22.)

Liu et al. and Bertozzi et al. are discussed above. Karlsson et al.'s teaching of the use of plasmon resonance to determine binding affinity is simply a disclosure of a well known use for plasmon resonance and does not, either alone or in combination with Liu et al. and Bertozzi et al., render Claims 28-34, 36, 46, and 48-53 obvious.

Reconsideration and allowance of Claims 28-34, 36, 46, and 48-53 are respectfully requested.

VII. 35 U.S.C. §103

The Examiner has rejected Claims 28-34, 36, 46, 48-50, 52, 53, and 63 under 35 U.S.C. §103 as unpatentable over Liu et al. *supra*, in view of Bertozzi et al. *supra*, and Licitra et al. *supra*.

According to the Examiner,

"[I]t would have been obvious to provide the public access to the data through publication as taught by Licitra et al., because Liu et al. teach a three-hybrid assay and Licitra teach a three-hybrid assay and provide the data obtained from the assay." (See, Office Action, page 24.)

Liu et al., Bertozzi et al., and Licitra et al. are discussed above. Applicants assert that Licitra et al. teach away from improving the linker in their hybrid ligand by suggesting that a better approach would be to "generate yeast strains that are more permeable without significantly affecting yeast viability". (See, Licitra et al., page 12820, right column.)

Therefore, the combination of Liu et al., Bertozzi et al., and Licitra et al. can not render obvious Claims 28-34, 36, 46, 48-50, 52, 53, and 63.

Reconsideration and allowance of Claims 28-34, 36, 46, 48-50, 52, 53, and 63 are respectfully requested.

VIII. 35 U.S.C. §103

The Examiner has rejected Claims 28-34, 36-37, 39, 40, 42, 46, 48-50, 52, and 53 under 35 U.S.C. §103 as unpatentable over Liu et al. *supra*, in view of Bertozzi et al. *supra*, and Zaharevitz et al., *Cancer Research*, 59: 2566-2569 (1999) ("Zaharevitz et al.").

According to the Examiner,

"[I]t would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the hybrid ligand of the three-hybrid method of Liu et al. to include the CDK inhibitors disclosed by Zaharevitz et al. as ligand B, because Liu et al. teach that ligand B may be selected from a small molecule library and Zaharevitz et al. teach that the kinase inhibitor is a small molecule." (See, Office Action, page 26.)

Liu et al. and Bertozzi et al. are discussed above. Applicants assert that the added disclosure of Zaharevitz et al. simply that the kinase inhibitor is a small molecule does not render Claims 28-34, 36-37, 39, 40, 42, 46, 48-50, 52 and 53 obvious.

Reconsideration and allowance of Claims 28-34, 36-37, 39, 40, 42, 46, 48-50, 52 and 53 are respectfully requested.

IX. 35 U.S.C. §103

The Examiner has rejected Claims 28-34, 36, 46, 48-50, 52, and 53 over Liu et al. *supra*, in view of Holt et al., WO 96/06097 ("Holt et al.").

According to the Examiner,

"It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the hybrid ligand in the three-hybrid assay to include the (CH₂-O-CH₂)_n linker of Holt et al. because Liu et al. teach it is within the ordinary skill in the art to use any linker known in the art and Holt et al. teach linkers for making homodimeric or heterodimeric ligands capable of forming a trimeric complex in a three hybrid assay." (See, Office Action, page 28.)

The Liu et al. document is discussed above. Holt et al. describe compounds that may be used to dimerize immunophilins (e.g., FKBP, which binds FK506). The compounds of Holt et al. contain a linker "L", but Holt et al. only generically suggest that the linker L "need not contain

essential elements for binding to the immunophilin proteins, and may be selected from a very broad range of structural types". (See, Holt et al., page 2, lines 22-23.) (emphasis added.) Although Holt et al. describe a cell-based transfection assay on pages 48-49, there is no relevant teaching in the Holt et al. citation as to which of the numerous types of disclosed linkers are preferred for any reason.

Also, there is no teaching in Holt et al. that would suggest that the PEG linkers would increase cellular uptake of the hybrid ligands despite its hydrophilic structure, as is demonstrated in the present application. As such, Holt et al. would not motivate one skilled in the art to select the subgenus of PEG linkers from the numerous linker genus disclosed therein. In addition, one skilled in the art would not have chosen a PEG linker-based construct when seeking to solve the technical problem of providing an improved membrane permeable heterodimeric hybrid ligand useful for *in vivo* application.

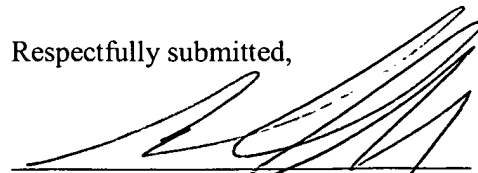
Therefore, the combination of Liu et al. with Holt et al. can not render Claims 28-34, 36, 46, 48-50, 52, and 53 obvious.

Reconsideration and allowance of Claims 28-34, 36, 46, 48-50, 52, and 53 are respectfully requested.

For the reasons set forth above, Applicants respectfully submit that the references cited by the Examiner in the present Office Action, considered together or individually, neither disclose nor suggest the novel method recited specifically in the present claims.

Reconsideration and withdrawal of the rejections to Claims 28-37, 39, 40, 42-46, 48-55, and 63 and allowance of all claims are respectfully requested.

Respectfully submitted,



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